

Final Report

Characterization of the Molecular Basis of Cell Recognition at Surfaces

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Introduction

In this work we have studied molecular recognition events that involve cell adhesion and the complex biological reactions involved phenomena such as healing the processing and storage of information. In particular we were interested in the dependence of nerve cells on the specific interactions with glial cells for proper function. Myelinating glial cells are thought to associate with neuronal axons, in part, via the cell-surface adhesion protein, myelin-associated glycoprotein (MAG). Related to this are a number of disorders associated with ganglioside metabolism, usually accompanied with severe symptoms such as mental retardation, blindness, and early mortality, notably Tay-Sachs disease, Sandhoff-Jatzkewitz disease and GM1 gangliosidosis.

The work for this project was done on site at the Naval Research Laboratories in collaboration with Dr. Gil Lee at NRL. Dr. Thomas Boland, a post-doctoral researcher, performed the work.

Our studies involved two levels. First, a fundamental level is essential for the understanding of the function of the specific ligand. Second, at a more practical level, we have developed strategies for incorporating gangliosides into a molecular biosensor, which will help early detection of associated disorders.

Of particular interest has been the relationship between interfacial structure and molecular recognition, with an emphasis on gangliosides. We have studied molecular interactions an atomic force microscope (AFM). Using specially modified tips we induced interactions with biomolecules adsorbed on a surface. In our measurements we have been able to measure the interaction forces in the piconewton range between the tip and surface and produce a two dimensional image of the surface forces. We have interpreted our results in terms of the relationship between the position of the probe and the intermolecular forces (electrostatic, long-range Van der Waals, hydrophobic/ hydrophilic steric/hydration forces). Correcting for artifacts observed with scanning probe techniques, significant information has been obtained at a molecular scale. For example analysis of mixed phospholipid/glycolipid bilayers has resulted in a clear understanding of the different forces involved.

Experimental

GM1 gangliosides have been incorporated into single phase and mixed phospholipid bilayers. The bilayers further were examined by using gold coated AFM tips functionalized with self-assembled alkanethiol monolayers (SAMS) terminated by methyl, hydroxyl and carboxylic acid functional groups.

We also developed strategies for immobilizing the gangliosides on the AFM tip. These include Langmuir-Blodgett (LB) deposition of a bilayer onto the AFM tip and self-assembly of on gold coated tips. The self-assembly strategy has been the most promising. Hetero bifunctional coupling agents were used to attach a carboxylic acid terminated thiol to an amine terminated oligoglycosyl (M. Sugimoto *et al.*, *Carbohydrate Research*, **156** c1-c5 (1986)). The resulting mercapto-glycosyl was then used to prepare pure and mixed self-assembled monolayers on

crystalline gold. The layers were analyzed by contact angle measurements, XPS, ellipsometry and infrared spectroscopy. These data showed that the coverage, thickness and structure of the monolayers were as expected. These strategies were then used to functionalize a series of AFM tips. These tips were used in turn to probe the phospholipid bilayers. In buffered solution, the gangliosides on tip and surface were shown to interact by hydrogen bonding between the hydroxyl and acetyl functions of the galactose and the specific binding interactions between the tip and surface were detected. The results demonstrate that such probes have potential use for ultra sensitive sensors, such as the force-amplified biosensor (FABS).

Conclusions

The results show several important issues concerning the structure function relationship of ligand-receptor pairs at molecular scales. We have demonstrated the building of supramolecular assemblies and have developed strategies for examining biological active molecules at the submonolayer level.

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14. ABSTRACT Molecular recognition interactions have been studied via atomic force microscopy using specially modified tips. Tip-surface interaction forces in the piconewton range were measured. From the from force-tip separation relationships significant information has been obtained about molecular scale interactions. In particular, analysis of mixed phospholipid/glycolipid bilayers has resulted in a clear understanding of the different forces involved. This work leads to a better understanding of molecular recognition events involving cell adhesion and complex biological reactions, such as healing, and has potential use in ultra sensitive biosensors.					
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